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Regional Variations and Adaptive Morphology of *Apis cerana* Forelegs Across India

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Authors' contributions

This work was carried out in collaboration among all authors. Authors MAV, RP, and PM conceptualized the study, developed the methodology, curated the data, conducted the investigation, performed formal analysis, and wrote the original draft. They also reviewed and edited the manuscript and contributed to the software used in the analysis. All authors read and approved the final manuscript.

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ABSTRACT

This study investigated the foreleg morphometry of *Apis cerana* across 16 diverse locations in India, spanning the northern, southern, and northeastern regions, to explore regional adaptations and environmental influences. The foreleg measurements were analyzed for five key segments: The coxa, trochanter, femur, tibia, and tarsus. Significant regional differences were observed, with northern populations exhibiting larger dimensions, southern populations showing smaller forelegs, and northeastern populations showing intermediate measurements. These variations are likely linked to the distinct climatic conditions and flora in each region, suggesting adaptive responses to environmental pressures. The tibia showed consistent dimensions across all regions, indicating its

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evolutionary conservation for basic leg functions, whereas the coxa and trochanter exhibited higher variation, reflecting fine-tuned adaptations to local ecological demands. This study provides valuable insights into the morphological diversity and functional adaptations of *Apis cerana*, contributing to our understanding of how environmental factors shape honeybee morphology.

Keywords: Apis cerana; foreleg morphometry; regional adaptations; environmental influences; tibia; coxa; trochanter; morphological diversity.

1. INTRODUCTION

The foreleg of a honeybee is a remarkable example of evolutionary adaptation, featuring several specialized structures essential for insect survival and daily activities. Located on the prothorax, each foreleg contains one of the bee, most distinctive features: the antennae cleaner, also known as the strigilis. This specialized structure consists of a curved, movable spur and a semi-circular notch lined with fine bristles, allowing the bee to meticulously clean its antennae by pulling them through this natural grooming device (Gana, 2018). Beyond the antennae cleaner, the foreleg is equipped with specialized eye brushes - fine bristles that help keep the bee's compound eyes free from pollen and debris. Like all insect legs, the honeybee's foreleg follows a segmented structure consisting of the coxa, trochanter, femur, tibia, and tarsus, that each segment powered by specific muscles enabling precise movements. These segments work in concert to support various critical activities, from basic locomotion to complex social behaviors such as waggle dancing. Through this intricate combination of structures, the honeybee's foreleg serves multiple functions: maintaining clean sensory organs, assisting in grooming, handling food and nest materials, and supporting balance during crucial activities such as foraging and communication within the colony (Rajak & Basavarajappa, 2016). Understanding these morphometric differences has become increasingly crucial in the context of climate change, which poses significant threats to biodiversity and ecosystem stability. By examining how *Apis cerana* has adapted to different ecological niches, this study aims to provide insights into the resilience and vulnerability of honeybees, contributing to strategies for their conservation and sustainable management in a changing environment.

In this study, aimed to analyze the foreleg characteristics of *Apis cerana* collected from 16 locations across India. These locations within the north, south, and northeast of India were selected to represent diverse geographic and ecological conditions. By examining specific foreleg traits, it is necessary to explore variations influenced by environmental factors and regional adaptations, enhancing deeper understanding of the morphological diversity and evolutionary adaptations of *A. cerana* across its distribution range.

2. MATERIALS AND METHODS

2.1 Sample Collection

A comprehensive study of *Apis cerana* workers bee collected from 16 different locations across India, including Andhra Pradesh, Assam, Cuddalore (Tamil Nadu), Gujarat, Hissar (Haryana), Karnataka, Manipur, Nagaland, Odisha, Palampur (Himachal Pradesh), Pantnagar (Uttarakhand), Punjab, Rajasthan, Srinagar (Jammu and Kashmir), Tamil Nadu, and West Bengal. These locations were strategically selected to encompass a wide range of latitudes, altitudes, and climatic conditions, ensuring a diverse representation of the species. Thirty bees were collected from the each location, and their forelegs were studied. This approach allowed us to investigate the geographic and ecological variations that influence the morphometric diversity of *A. cerana*, providing valuable insights into species differentiation and regional adaptations (Andere et al., 2008).

2.2 Morphometric Analysis

The dissection and morphometric study of honeybee forelegs requires careful preparation and precise execution following a systematic protocol. Initially, all necessary materials including fresh honeybee specimens, dissection microscope, fine forceps, scissors or scalpel, glass slides, coverslips, petri dishes, physiological saline solution, and glycerin were used. The dissection process began by anesthetizing fresh specimens, followed by placing them in a dissecting dish containing saline solution. Under microscopic guidance, was carefully hold and the bee's thorax and the foreleg was gently detached the coxal joint, ensuring complete removal of the leg without damaging its segments. The dissected leg should then be cleaned of attached tissues and mounted on a glass slide using glycerin and covered with a coverslip for detailed examination. The morphometric study involves comprehensive measurements and analysis of various structural components. Begin by measured the total leg length and individual segment lengths including the coxa, trochanter, femur, tibia, and all five tarsal segments.

2.3 Statistical Analysis

The foreleg characteristics across India were subjected to statistical analyses using Principal Component Analysis (PCA) and cluster analysis. These methods were employed to identify patterns and groupings within the data, highlighting variations and similarities among populations from diverse geographic and ecological regions. PCA was used to reduce the dimensionality of the dataset while retaining the most significant traits contributing to variability. Cluster analysis was performed to group the populations based on their morphological similarities, providing insights into potential subspecies differentiation and regional adaptations. This integrated approach ensures a comprehensive understanding of the morphometric diversity of *A. cerana*.

3. RESULTS

3.1 Regional Variations in Foreleg Morphometry

The study examined five key segments - coxa, trochanter, femur, tibia, and tarsus - across sixteen different locations spanning northern, southern, and northeastern India. The data shows distinct regional patterns, with northern Indian populations (including Srinagar, Palampur, Punjab, Hissar, and Pantnagar) consistently displaying larger measurements with an average total length of 4.854 mm, while southern populations (Tamil Nadu, Karnataka, Andhra, and Cuddalore) exhibited smaller dimensions averaging 4.757 mm. The northeastern populations (Assam, Manipur, and Nagaland) demonstrated intermediate measurements with an average of 4.823 mm, reflecting their unique ecological position. Fig. 1 illustrates the hierarchical analysis of foreleg measurements from different regions of India, while Fig. 2 presents the cluster analysis, highlighting regional groupings. Table 1 provides the detailed foreleg characteristics across various Indian regions, and Table 2 shows the eigenvalues of the correlation matrix used in the analysis.

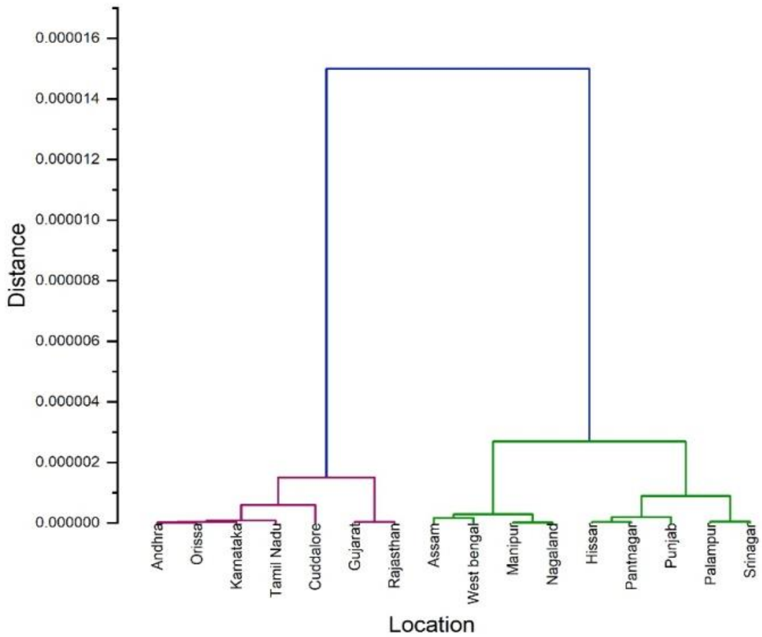

Fig. 1. Hierarchal Analysis of foreleg from different regions of India

Table 1. Foreleg characteristic from different region from India

Location	Coxa	Trochanter	Femur	Tibia	Tarsus	Total
Andhra Pradesh	0.399 ± 0.01	0.463 ± 0.017	1.197 ± 0.011	1.504 ± 0.017	1.197 ± 0.018	4.76 ± 0.017
Assam	0.412 ± 0.02	0.476 ± 0.018	1.212 ± 0.012	1.5169 ± 0.01	1.202 ± 0.019	4.818 ± 0.018
Cuddalore	0.396 ± 0.02	0.46 ± 0.1777	1.195 ± 0.014	1.504 ± 0.018	1.195 ± 0.017	4.75 ± 0.011
Gujarat	0.403 ± 0.04	0.467 ± 0.016	1.203 ± 0.017	1.508 ± 0.019	1.201 ± 0.016	4.781 ± 0.019
Hissar	0.416 ± 0.04	0.484 ± 0.016	1.22 ± 0.018	1.523 ± 0.019	1.207 ± 0.019	4.85 ± 0.012
Karnataka	0.399 ± 0.05	0.462 ± 0.017	1.197 ± 0.017	1.504 ± 0.017	1.197 ± 0.014	4.759 ± 0.015
Manipur	0.412 ± 0.08	0.478 ± 0.017	1.214 ± 0.014	1.518 ± 0.0188	1.202 ± 0.011	4.824 ± 0.014
Nagaland	0.412 ± 0.07	0.479 ± 0.011	1.215 ± 0.016	1.519 ± 0.017	1.203 ± 0.012	4.828 ± 0.018
Orissa	0.4 ±0.09	0.463 ± 0.017	1.198 ± 0.019	1.505 ± 0.018	1.198 ± 0.015	4.764 ± 0.017
Palampur	0.418 ± 0.07	0.489 ± 0.057	1.221 ± 0.011	1.523 ± 0.016	1.208 ± 0.016	4.859 ± 0.019
Pantnagar	0.416 ± 0.010	0.483 ± 0.017	1.219 ± 0.014	1.522 ± 0.014	1.207 ± 0.015	4.847 ± 0.015
Punjab	0.417 ± 0.017	0.485 ± 0.051	1.221 ± 0.0189	1.522 ± 0.011	1.208 ± 0.018	4.853 ± 0.013
Rajasthan	0.404 ± 0.018	0.468 ± 0.0471	1.204 ± 0.016	1.509 ± 0.012	1.201 ± 0.019	4.786 ± 0.018
Srinagar	0.418 ± 0.014	0.489 ± 0.071	1.223 ± 0.0178	1.524 ± 0.013	1.209 ± 0.017	4.863 ± 0.016
Tamil Nadu	0.398 ± 0.015	0.462 ± 0.071	1.197 ± 0.0198	1.504 ± 0.014	1.197 ± 0.019	4.758 ± 0.013
West bengal	0.41 ± 0.017	0.476 ± 0.071	1.211 ± 0.0165	1.516 ± 0.017	1.202 ± 0.015	4.815 ± 0.011
C.D	2.25	1.82	2.35	2.65	2.74	4.25

Table 2. Eigenvalues of the correlation matrix

	Eigenvalue	Percentage of Variance	Cumulative
1	4.82867	96.57%	96.57%
2	0.10433	2.09%	98.66%
3	0.02814	0.56%	99.22%
4	0.02277	0.46%	99.68%
5	0.01609	0.32%	100.00%

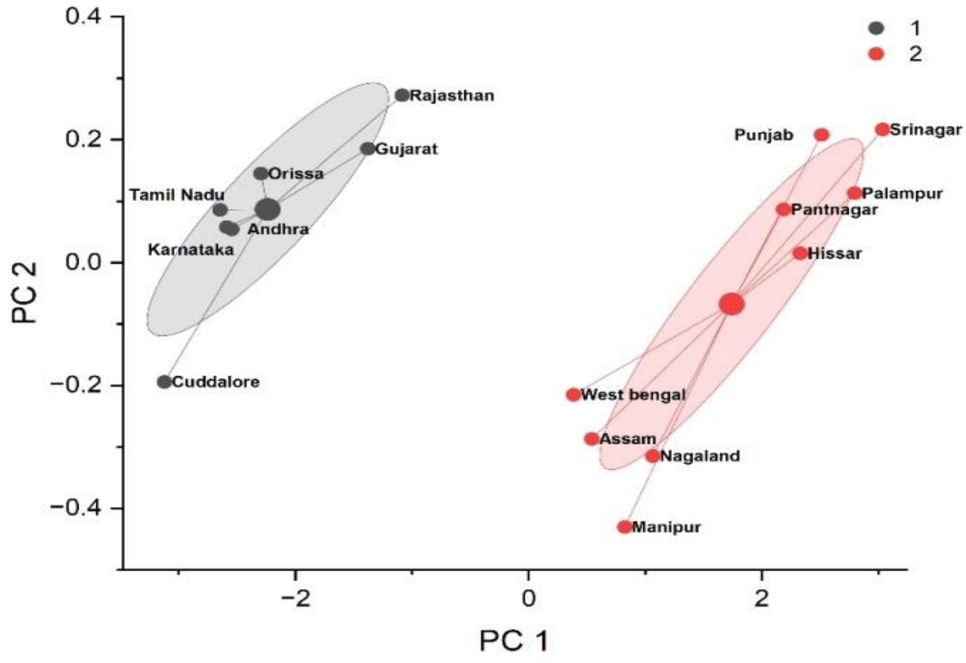

Fig. 2. Cluster analysis of foreleg from different regions of India

3.2 Detailed Segmental Morphometric Analysis of Forelegs

In the detailed segment analysis, the coxa measurements ranged from 0.396 to 0.418 mm, with Palampur and Srinagar showing the largest dimensions and Cuddalore the smallest. The trochander varied from 0.460 to 0.489 mm, following a similar pattern. The femur measurements ranged from 1.195 to 1.223 mm, with Srinagar exhibiting the largest and Cuddalore the smallest dimensions. Tibia measurements ranged from 1.504 to 1.524 mm, showing the highest consistency across populations while maintaining the north-south gradient. The tarsus segment varied from 1.195 to 1.209 mm, with Srinagar again showing the largest measurements and Cuddalore the smallest. These variations created a total length difference of 0.113 mm between the largest and smallest specimens, representing significant morphological adaptation.

4. DISCUSSION

4.1 Comparison with Existing Morphometric Data

The Marghitas *et al*., *Apis cerana* workers as being approximately 8 mm long, with fore leg ranging from 7.4 to 9.0 mm. The foreleg lengths measured in our study (ranging between 4.75 to 4.863 mm across locations) fit well within the expected proportionality for a 10 mm-long worker bee. This suggests that our measurements are consistent with the morphological characteristics of *Apis cerana* (Mărghitaş et al., 2008; Deka et al., 2011 & Schönitzer, 1986).

4.2 Environmental Adaptations of Honeybee Forelegs in India

In India, the forelegs of the Indian honeybee (*Apis cerana*) exhibit remarkable adaptations to the subcontinent's diverse climate and flora, aligning with the varied environmental conditions from the Himalayas to the coastal regions. The consistently larger measurements found in the northern populations, particularly in Srinagar (total length 4.863 mm) and Palampur (4.859 mm), likely represent adaptations to higher altitudes and colder climates. These larger dimensions could provide several adaptive advantages: enhanced muscle mass for better thermoregulation, improved stability in stronger mountain winds, and better handling of distinct highland flora. The increased size might also facilitate more efficient collection of pollen from the unique high-altitude flowers that characterize these regions (Du et al., 2019).

4.3 Climate-Driven Adaptations in Forelegs

In contrast, the southern populations, exemplified by Cuddalore (4.750 mm) and Tamil Nadu (4.758 mm), showed consistently smaller measurements across all the segments. This reduction in size could be an adaptation to warmer climates, potentially offering better heat dissipation through a higher surface-area-to-volume ratio. The smaller dimensions might also reflect adaptations to the predominantly tropical flora of southern India, where smaller, more delicate floral structures might require less robust handling apparatus.The intermediate measurements observed in the northeastern populations (Assam 4.818 mm, Manipur 4.824 mm, Nagaland 4.828 mm) are particularly interesting because they suggest a unique adaptive response to the region's distinctive climate and flora. These intermediate values might represent an optimal compromise between the requirements for handling diverse subtropical flora and dealing with variable climatic conditions characteristic of this region (Ebru et al., 2023 & Mahwish et al., 2018).

4.4 Segment-specific Functional Adaptations in Forelegs

Segment-specific variations provide additional insights into functional adaptations. The tibia, showing relatively consistent proportions across populations (range 1.504-1.524 mm), suggests strong evolutionary conservation of this segment's functional role, possibly due to its critical importance in basic leg mechanics and pollen handling (Rehman et al., 2018). Conversely, the higher relative variation in coxa and trochanter measurements might indicate their role in fine-tuning leg function to meet local environmental demands (Hongyi et al., 2018). These findings align with Bergmann's rule, which suggests larger body sizes in colder climates, although applied at a more localized scale (Peter et al., 2010 & Ajao et al., 2014). The consistent gradients in the measurements also support the concept of clinal variation in response to environmental gradients, a well-documented phenomenon in insect populations (Mayekar et al., 2022).

5. CONCLUSION

This study highlights significant regional and segmental variations in the foreleg morphology of *Apis cerana* across diverse locations in India, thereby important ecological and evolutionary adaptations. The larger foreleg dimensions observed in northern populations suggest adaptations to colder, high-altitude climates, whereas the smaller foreleg measurements in southern populations may reflect adaptations to warmer, tropical environments. Intermediate measurements in the northeastern populations likely represent a balance between climatic and floral factors. The consistency in tibia measurements across regions suggests evolutionary conservation of basic leg functions, while the variation in coxa and trochanter dimensions underscores the role of fine-tuning leg functionality to local conditions. These findings provide valuable insights into the adaptive evolution of *Apis cerana*, and demonstrate how morphological traits are shaped by regional environmental pressures.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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